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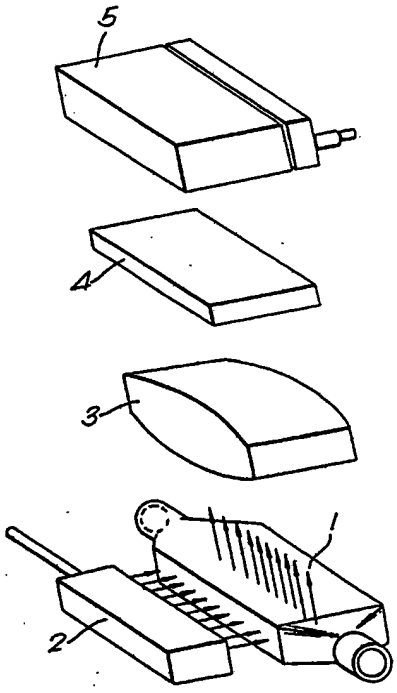
(54) Title: PROCESS AND APPARATUS FOR COUNTING PARTICLES

(57) Abstract

Particles in a fluid are counted by passing the fluid through an optical cell and an image of said particles is projected onto an array of charge coupled devices such that the area of the image of each particle at the said array is approximately the same as the area of at least a single charge coupled device, and signals from the individual charge coupled devices are processed to provide information concerning at least the number of said particles passing through the optical cell. A particle counter disclosed comprising an optical cell (1) through which is passed a fluid containing particles to be counted, means (2) for illuminating the particles in the optical cell and optical means (3) for providing an image of the particles on an array of charge coupled devices (5) such that the area of the image of each particle at the said array is approximately the same as the area of at least a single charge coupled device. The particles may fluoresce and a transmittance filter (4) may be provided.



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Process and apparatus for counting particles.

This invention concerns a method and apparatus for counting particles.

Existing particle counters, for example for counting microbial cells, are cumbersome and expensive devices commonly employing a significant power source either to maintain a high voltage field as in the Coulter system or laser illumination as in the conventional flow cytometer. It would be convenient in many cases to be able to count particles accurately in the field using a system readily capable of transportation and using a portable power source, e.g. batteries.

The present invention is based on the concept of using an array of charge coupled devices (CCDs) as the counting device. It is proposed, to project an image of the particles onto the CCD array such that the area of the image of an individual particle is approximately the same as the area of at least one CCD. However, in conventional, commercially available CCD arrays (chips) the area of each CCD, although very small, is larger than that of a typical microbial or other cell or cell nucleus or of many other particles, such as dust particles, which may require to be counted. Thus a preferred form the invention includes projecting a magnified image of the particle. This avoids the problem that a single CCD will provide a signal deriving from several particles simultaneously, thus preventing the possibility of counting individual particles.

The term CCD array as used herein refers to an array of photosensitive CCDs which may for example, be of the frame-field or interline transfer type and may produce the required signals by current or voltage sensing. Such arrays are normally provided as integral CCD chips for use, for example, in solid state cameras

and one commercially available CCD chip is that available from Phillips N.V.

According to the present invention therefore we provide a method of counting particles in which a fluid containing said particles is passed through an optical cell and an image of said particles is projected onto an array of charge coupled devices such that the area of the image of each particle at the said array is approximately the same as the area of at least a single charge coupled device, and signals from the individual charge coupled devices are processed to provide information concerning at least the number of said particles passing through the optical cell.

According to a further feature of the invention we provide a particle counter comprising an optical cell through which is passed a fluid containing particles to be counted, means for illuminating the particles in the optical cell and optical means for providing an image of the particles on an array of charge coupled devices such that the area of the image of each particle at the said array is approximately the same as the area of a least a single charge coupled device.

The diameter of each CCD in the array will commonly be in the range 5-10 microns e.g. 6-7 microns. This is of the same order as many particles which may advantageously be counted, for example cell nuclei.

The image projected on to the CCD array is preferably magnified. The magnification of the image of the particles may be in the range 2 to 15, preferably 5 to 10. If necessary, additional lenses may be moved in and out of the optical path or may be moved therein to change the magnification.

It is possible to derive the number of particles in the fluid using merely a strip-like array of CCDs having only 2 or 3 CCDs in the direction of fluid flow but it is much better to use a larger array of CCDs to provide the possibility of as many as 10 'pictures' of each

particle as its image moves across the CCD array e.g. a minimum of 30 rows of CCDs; this permits greater statistical analysis of the information and much greater accuracy of counting, as well as the possibility of providing information on particle size distribution. In general, it is preferred to use a conventional CCD chip, comprising of the order of 412 x 419 CCDs.

The signals from the CCD array can be processed by a computer, which may be a relatively unsophisticated personal computer, using appropriate software.

The software is designed to interrogate the CCDs as small groups or clusters, for example 3 x 3 CCDs. In any such group of 3 x 3 CCDs, it will be appreciated that if the size of the particle image is at least of the same order as one CCD, a number of CCDs will be at least partially covered by the image of the particle and provide a signal while the remainder will indicate the absence of the image. This enables the coordinates and boundaries of the image on the CCD array to be established. The particle concentration or the dimensions of the particle stream will be adjusted to ensure adequate spacing of the particles. The information provided by the CCDs can give an indication of particle size as well as position. As the images of the particles traverse the CCD array, the information concerning each particle can be checked several times. Sampling will generally be of the order of 50 times per second. If the particle stream is found to be moving too fast for accurate counting, the speed may be controlled, for example by feedback of information from the CCDs.

It may be desirable to count only particles reflecting or emitting light of a particular colour or to eliminate incidental light unrelated to the particles and it is then desirable to include a filter between the optical cell and the CCD array. Thus, in counting cells or cell-nuclei stained with a fluorescent dye, it may be

desirable to use a filter transmitting only light of the required wavelength. This avoids counting any unstained particles and also cuts out any of the radiation used to illuminate the particles in such a fluorescent system.

It is also possible to count more than one population of cells simultaneously if these are sufficiently differentiated either by size and/or colour. In the case of colour differentiation, it is necessary to use more than one transmittance filter which may, for example, be mounted on a rotating device introducing the filters successively into the light path, possibly separated by light obstructing sections so that light of different colours will be intermittently projected onto the CCD array. The rotation of the above device can readily be synchronised with the signal processing system to distinguish the images produced by the differently coloured particles.

The optical cell will normally be illuminated by radiation incident approximately perpendicular to the light path from the cell to the CCD array. This maximises the contrast between the particle image and the background. It is particularly preferred to use devices according to the invention to count particles stained with a fluorescent dye, in which case, the side-illumination can be a source of appropriate radiation, e.g. ultra-violet light. Where visible light is used simply to illuminate the particles, colour differentiation may be assisted by interposing transmittance filters between the light source and the cell.

Data processed by a computer connected to the chamber may be displayed, for example on a LCD display (particularly where battery power only is desirable) and can give not only particle numbers but a histogram or other representation of size and/or colour distribution. A printer, e.g. a thermal printer, may be attached to provide a hard copy print-out of the information.

The optical cell may be of design conventional for cell counting. It is preferred that the flow path is very thin so that only a single layer of particles is counted and overlap is minimised. The width of the cell will be such that the image of the cells on the CCD array falls within the dimensions of the array. Alternatively, the image falling on the CCD array can be derived solely from a known restricted area of the cell comprising a fixed fraction of the width of the cell, so that only a known proportion of the particles is counted. Typically, for biological cell counting, the dimensions of the optical cell may be: thickness 0.1 mm x width 4 mm x length 4 mm, thickness being the dimension perpendicular to the optical path of the imaging system.

However, use of high quality, short focal length optical system for projecting an image of the particles onto the CCD array enables a thin slice of the fluid to be visualised selectively. The volume of the slice can be determined and will effectively comprise the volume of fluid subjected to particle counting. This provides a simple alternative to the use of laser light in conventional cytometers to provide and accurate thin layer of illumination. Lasers require a power supply which cannot be provided by batteries even on an intermittent basis whereas, as indicated above, it is an advantage of the devices of the present invention that they can be powered solely by batteries and may thus be used when an electrical mains or power source is unavailable.

The system and device according to the invention are particularly useful in conjunction with the magnetic particle separation device according to our co-pending PCT application claiming priority from GB 8927744.6, filed 7 December 1989. This enables superparamagnetic particles to be attached to analytes including biological cells and readily separated from reagents and

unwanted contaminants (such as cells other than those of interest) before being passed to the particle counting device. This enables cells in a diverse population, e.g. a blood sample, readily to be counted, and provides valuable tool for diagnosis of diseases. Generally such a separation device may comprise a chamber provided with opposed electromagnets which can be energised in turn. Energisation of the first magnet causes magnetic particles within the chamber to aggregate onto the chamber wall near that magnet. After removal of supernatant and introduction of a further fluid, the particles may be re-dispersed, by energising the other magnet and causing the particles to move across the fluid to the other wall. This procedure can be repeated to complete that particular process stage and, with the particles aggregated on the wall, the fluid may be removed. Such a chamber may be provided with a fluid supply system whereby the sample and successive wash and reagent fluids may be introduced into the chamber via appropriate tubing by pressure transfer. This can readily be automated using electrically controlled pinch valves to permit transfer of an appropriate volume of each individual solution.

The magnetic particles are typically attached to selected cells via specific monoclonal antibodies bound to the surface of the magnetic particles. After processing, it may be more convenient to lyse the cells and count only the nuclei which have previously been stained by a fluorescent dye. However, whole cells may be passed to the particle counter with magnetic particles attached since these are very much smaller than the cells and will not interfere with counting.

Such magnetic particles may be used to bind to analytes other than cells and then, for example in a sandwich assay, labelled with an optical label e.g. fluorescent dye. The magnetic particles, after removal of unreacted label, may then be passed to the counter

together with unreacted magnetic particles, which can be distinguished by their lack of attached fluorescent label.

The invention will now be described by way of illustration only with reference to Figure 1 of the accompanying drawings which shows schematically a particle counting device according to the invention.

In the device shown in Figure 1, an optical cell 1 (0.1 mm x 4 mm x 4 mm) is illuminated on one side by an ultraviolet light source 2. A magnifying optical system, schematically represented by the lens 3, is positioned in a light path perpendicular to the incident UV light from the source 2 and a transmittance filter 4 is also positioned in the light path. A CCD array 5, typically a CCD chip (9 x 6 mm) is provided at the end of the light path and receives a magnified image of particles in the counter focused onto the surface of the CCDs. By appropriate focusing, light can be selected from a layer or slice of the interior of the optical cell 1, the volume of this slice being known or calibrated so that the number of particles per unit volume of the fluid may be calculated.

The cell 1 typically is made from optical quality glass although optical plastics may be used. The shape of the cell is designed to fit the optical lens system 3 and the CCD chip 5. The lens system has a magnification ratio that will make a normal particle (e.g. a nucleus) cover up to 3 x 3 = 9 CCDs in the CCD chip 5. By choosing this ratio, the analyzer facilitates measurements of both smaller and larger particles than normal. The CCD chip is chosen on the basis of its CCD density, sensitivity and reaction time.

Since the fluorescent dye, e.g. acridine orange, has a definite wavelength for its emmission, the filter 4 is placed between the lens system 3 and the CCD chip 5 to filter out light of other wavelengths.

The CCD chip gives continuous information regarding

what is happening in its viewing field. This information is transmitted to the processing unit in the analyzer (not shown).

The analyzer includes a computer (not shown) which handles all communication with the operator and all data analysis. Communication with the operator is via a conventional touch sensitive panel, such as a keyboard, and an LCD screen (neither of which are shown). At the beginning of the analysis the computer will tell the operator to check certain functions and how to insert the sample. The processor will control the valve function and monitor the pressure in the fluid system. The energisation of the magnetic fields is controlled and the data from the CCD chip 5 is collected.

The information from the CCD chip may, for example, be sampled as 10 "frame freeze pictures", because the information from the CCD chip will be real time information from a moving fluid. The information from the 10 pictures will be processed by appropriate imaging software capable of identifying the number of illuminated objects and the size distribution.

The data obtained from the 10 pictures is treated statistically and the results for the test are calculated.

The results of the analysis are presented on the LCD screen in terms of the number of cells and a histogram of the size distribution although other presentations will be apparent to the skilled person. The latter will give the operator a chance to see if the sample contained so many abnormal cells that further study is necessary.

A printer, e.g. a thermal printer, may be provided to print out a hard copy of the test results.

Claims

1. A method of counting particles in which a fluid containing said particles is passed through an optical cell and an image of said particles is projected onto an array of charge coupled devices such that the area of the image of each particle at the said array is approximately the same as the area of at least a single charge coupled device, and signals from the individual charge coupled devices are processed to provide information concerning at least the number of said particles passing through the optical cell.

2. A method of counting particles as claimed in claim 1 wherein the image of said particles is projected onto the array of charged coupled devices by magnifying lens means.

3. A method of counting particles as claimed in claim 2 wherein the lens means has a short focal length.

4. A method of counting particles as claimed in any one of claims 1 to 3, wherein said particles are illuminated approximately perpendicular to a light path from the optical cell to the array of charged coupled devices.

5. A method of counting particles as claimed in any preceding claim wherein the particles fluoresce.

6. A method of counting particles as claimed in any preceding claim wherein at least one transmittance filter is disposable between the optical cell and the array of charged coupled devices.

7. A method of counting particles as claimed in any preceding claim wherein the array of charged coupled

devices are interrogated in clusters, the devices in each cluster being interrogated substantially simultaneously.

8. A particle counter comprising an optical cell through which is passed a fluid containing particles to be counted, means for illuminating the particles in the optical cell and optical means for providing an image of the particles in an array of charge coupled devices such that the area of the image of each particle at the said array is approximately the same as the area of a least a single charge coupled device.

9. A particle counter as claimed in claim 8 comprising optical means which provide a magnified image of the particles on the array of charged coupled devices.

10. A particle counter as claimed in claim 8 or claim 9 comprising optical means having a short focal length.

11. A particle counter as claimed in any one of claims 8 to 10, wherein the means for illuminating the particles provides radiation approximately perpendicular to a light path from the optical cell to the array of charged coupled devices.

12. A particle counter as claimed in any one of claims 8 to 11 comprising at least one transmittance filter disposable between the optical cell and the array of charged coupled devices.

13. A particle counter as claimed in any one of claims 8 to 12 further comprising means for processing signals from the individual charge coupled devices to provide information concerning at least the number of said particles passing through the optical cell.

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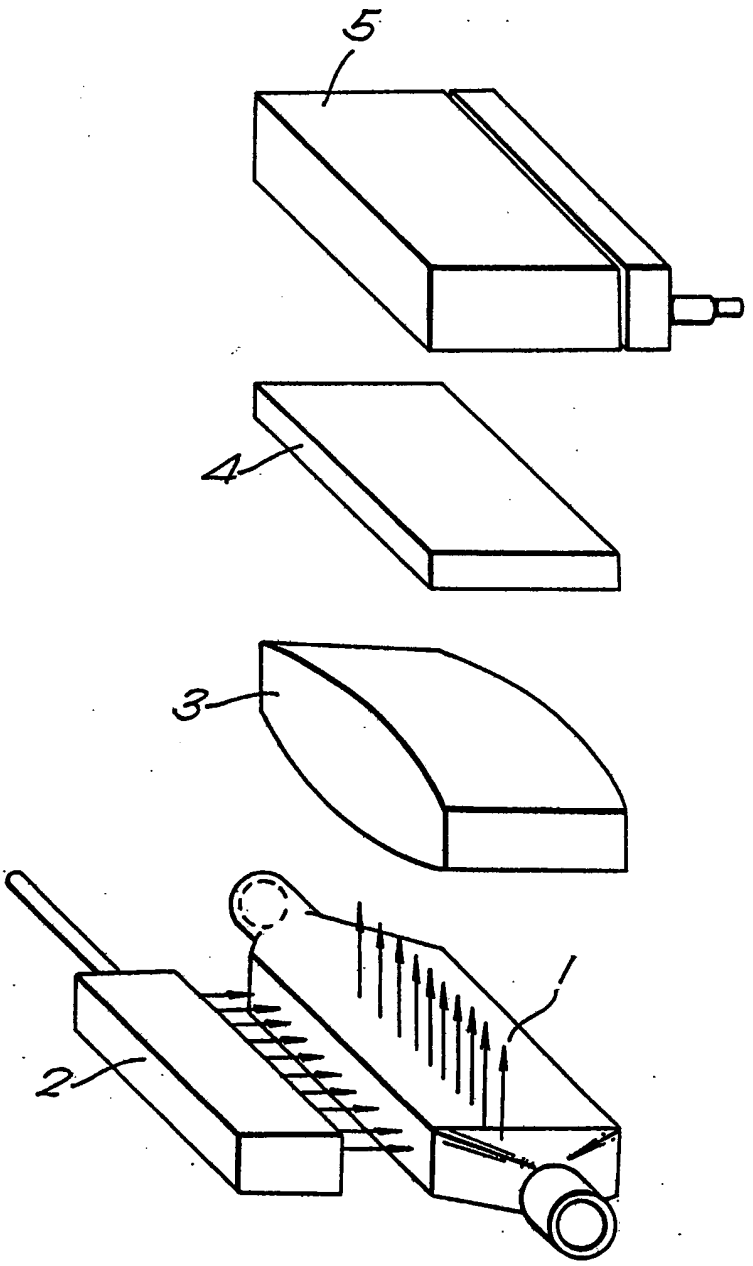

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9002121 SA 42213

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EP-A- 0195420	24-09-86	DE-A- 3510363	25-09-86
		JP-A- 61223632	04-10-86
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